Effect of Tumor Necrosis Factor Alpha and Transforming Growth Factor Beta 1 on Plasminogen Activator Inhibitor-1 Secretion From Subcutaneous and Omental Human Fat Cells in Suspension Culture

Helga Gottschling-Zeller, Michael Birgel, Karin Röhrig, and Hans Hauner

Elevated levels of plasminogen activator inhibitor-1 (PAI-1) are characteristic of the obese state and may contribute to the association between obesity and cardiovascular disease. In this study, we measured the rate of secretion of PAI-1 antigen in isolated subcutaneous and omental abdominal adipocytes from severely obese and non-obese individuals and studied the effect of selected cytokines on PAI-1 release using a suspension culture technique. PAI-1 secretion was approximately 2-fold greater in isolated fat cells from the obese versus non-obese subjects. In addition, PAI-1 mRNA levels were higher in adipose tissue samples from obese versus non-obese individuals ($P < .05$). PAI-1 release was also approximately 2-fold greater in omental versus subcutaneous adipocytes from both obese and non-obese subjects (each $P < .05$). A 24-hour exposure to 1 nmol/L tumor necrosis factor alpha (TNF-α) slightly increased PAI-1 release from both subcutaneous and omental adipocytes (30% ± 21% and 17% ± 18%, respectively, nonsignificant [NS]). Transforming growth factor beta 1 (TGF-β1) induced a significant dose-dependent increase of PAI-1 release into the medium. Exposure to 400 pmol/L TGF-β1 of subcutaneous and omental fat cells from both obese and non-obese individuals elevated PAI-1 secretion by 2-fold. These data provide evidence that human fat cells release a substantial amount of PAI-1 in a depot-specific manner and that TGF-β1 particularly contributes to the regulation of PAI-1 secretion.

OBESITY is associated with many diseases, particularly if the excess body fat is preferentially located in the abdominal region. An abdominal pattern of fat distribution is a characteristic component of the insulin resistance syndrome which is a predisposing condition for type 2 diabetes and coronary heart disease.[1] Among the multiple mechanisms to explain the relationship between obesity and cardiovascular disease, disorders of the fibrinolytic system seem to play an important role. In particular, there is growing evidence that increased levels of plasminogen activator inhibitor-1 (PAI-1) favor the development of thromboembolic complications.[2] PAI-1 is a member of the family of serpins (serine protease inhibitors) and the main regulator of the endogenous fibrinolytic system by inhibiting tissue-type plasminogen activator.[3,4]

Elevated plasma levels of PAI-1 have been found in obese individuals in many clinical and epidemiological studies[2,5] and appear to be particularly correlated with an abdominal distribution of adipose tissue in both men[6] and women,[7-10] but are also positively associated with other components of the insulin resistance syndrome.[2,5,9-12] The mechanisms responsible for the elevated PAI-1 plasma levels in this syndrome are not yet completely elucidated.

In vitro studies have demonstrated that a variety of cell types are able to produce PAI-1 protein.[13-16] In 1991, Sawdey and Loskutoff[13] first demonstrated the expression of PAI-1 in mouse adipose tissue. Subsequent studies detected PAI-1 mRNA and protein in visceral and subcutaneous fat from obese rats[17] and in differentiated 3T3-L1 adipocytes.[18,19] Recently, the expression and secretion of PAI-1 has also been demonstrated in adipose tissue samples from humans.[20,21] Little is currently known about the regulation of PAI-1 in adipose tissue. Tumor necrosis factor alpha (TNF-α), which is overexpressed in obesity,[22] is a potential stimulator of PAI-1 production.[13,18,23,24] However, another cytokine, transforming growth factor beta 1 (TGF-β), appears to be the major inducer of PAI-1 synthesis in adipose tissue.[13,19,20,24] Alessi et al[20] recently described a stimulatory effect of TGF-β1 on PAI-1 production in human adipose tissue explants.

The aim of this study was to compare PAI-1 expression and secretion in omental and subcutaneous adipocytes from obese and non-obese subjects in suspension culture. Moreover, we were particularly interested in the effect of TNF-α and TGF-β1 on PAI-1 secretion in order to obtain additional information on a possible site-specific regulation of PAI-1 production.

MATERIALS AND METHODS

Materials

Collagenase CLS type 1 was obtained from Worthington (Freehold, NJ). Human insulin and cortisol were kindly donated by Hoechst (Frankfurt, Germany). Culture media were obtained from GIBCO/BRL (Berlin, Germany). Human TGF-β1 and gentamycin were purchased from Sigma (Munich, Germany), and TNF-α was from Pepro Tech (Rocky Hill, NJ). The PAI-1 enzyme-linked immunosorbent assay (ELISA) kit was purchased from WAK Chemie (Hamburg, Germany). Superscript RT reverse transcriptase, random hexamers, Taq polymerase, polymerase chain reaction (PCR) buffer, dCTP, dGTC, dTTP, and dATP were obtained from GIBCO/BRL. ^{33}P-dATP was obtained from Amersham (Braunschweig, Germany). For sequencing, we used the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit from Perkin Elmer (Weiterstadt, Germany). All other chemicals were from Boehringer (Mannheim, Germany) or Merck (Darmstadt, Germany). Sterile plasticware for tissue culture was purchased from Flow Laboratories (Irvine, Scotland).

Subjects

Subcutaneous and omental adipose tissue samples (2 to 10 g wet weight) were obtained from 11 extremely obese individuals (8 females and 3 males with a body mass index [BMI] >40 kg/m^2) who underwent vertical gastric banding for weight reduction. Two patients had type 2 diabetes mellitus that was treated with diet and metformin. Except for

From the Clinical Department, Diabetes Research Institute, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany.

Submitted June 25, 1999; accepted October 25, 1999.

Address reprint requests to Hans Hauner, MD, Diabetes Research Institute, Heinrich-Heine-University Düsseldorf, Auf'm Hennekamp 65, D-40225 Düsseldorf, Germany.

0026-0495/00/4905-0005$10.00/0

these patients, the other obese subjects were healthy and did not use any medication. In addition, we also obtained adipose tissue samples from 7 never-obese subjects (2 females and 5 males) who served as a control group. All subjects were of caucasian origin and did not have acute infection, cancer, or any other consuming disease. Clinical characteristics of the patients are summarized in Table 1. All patients provided informed consent to participate in the study. The procedure for obtaining human adipose tissue was approved by the Ethics Committee of Heinrich-Heine-University, Düsseldorf.

Isolation and Culture of Fat Cells

Immediately after obtaining adipose tissue, the samples were transported to the laboratory in Dulbecco's modified Eagle's medium/Ham's F12 medium (DMEM/F12) containing 4% bovine serum albumin (BSA). Fat cells were isolated by collagenase digestion according to the method of Rodbell.[25] Briefly, the fat specimens were cut into small pieces (1 to 2 mm^2) under sterile conditions and incubated for 45 minutes in a shaking water bath at 37°C in a collagenase solution (1.0 mg collagenase/mL phosphate-buffered saline [PBS] also containing 4% BSA). After enzymatic disaggregation of the tissue, the floating adipocyte layer was washed 3 times with PBS buffer with 4% BSA. Finally, the undigested tissue was removed by filtration through a nylon mesh of 280-μm pore size. After isolation, fat cell size and fat cell number were determined in diluted aliquots. The assessment of the mean fat cell diameter of the samples was based on at least 150 cells from each adipose tissue sample. The mean fat cell volume was calculated from the diameter. The freshly isolated fat cells were incubated for the time indicated (usually 24 hours) in DMEM/F12 supplemented with 4% BSA, 66 nmol/L insulin, 100 nmol/L cortisol, and 50 μg/mL gentamycin at 37°C in a shaking water bath at a dilution of 1:10. The culture medium was then collected and immediately stored at −80°C until later measurement of the PAI-1 protein concentration. In separate experiments, TGF-β1 (4, 40, or 400 pmol/L) or 1 nmol/L TNF-α were added to the medium.

For the assessment of fat cell viability, the level of lactate dehydrogenase (LDH) was measured in the culture medium at the beginning of the experiment and at 2, 6, and 24 hours of incubation. There was no significant change in LDH activity per hour during the 24-hour period (data not shown). Furthermore, the mean fat cell diameter was determined before and after 24 hours of suspension culture to be without significant alteration (data not shown).

Measurement of PAI-1 Protein

The PAI-1 antigen concentration was determined in the culture medium using a commercially available specific ELISA kit. The interassay and intraassay variation was less than 10% and 5%, respectively.

RNA Extraction

Total RNA was extracted according to the method described by Chomczynski and Sacchi.[26] In a subgroup of 14 subjects, there was sufficient adipose tissue for isolation of RNA. These samples were immediately added to a 4-mol/L guanidine thiocyanate buffer supplemented with 0.7% mercaptoethanol (5 mL/g wet tissue), homogenized using an Ultraturrax (Ika Labortechnik, Staufen, Germany), and stored at −80°C for later RNA preparation. For this purpose, the homogenate was mixed with chloroform/isoamyl alcohol, water-saturated phenol, and sodium acetate. After centrifugation, the aqueous phase was mixed with an equal volume of isopropyl alcohol. After 12 hours at −20°C, the RNA was pelleted for 15 minutes at 10,000 × *g*, washed twice with 70% ethanol, dried, and redissolved in Tris-EDTA buffer, pH 7.5. The amount and purity of the RNA yield was verified by electrophoresis in a formaldehyde-containing agarose gel. Total RNA was dissolved to 0.2 μg/μL in Tris-EDTA buffer, and first-strand cDNA was prepared using 5 μL RNA, Superscript RT reverse transcriptase, and random hexamers according to the instructions of the manufacturer. cDNA was diluted 1:8 with H_2O, and PCR was performed using 7 μL diluted cDNA and a PCR primer mix containing 1.0 U Taq polymerase in its 1× buffer, 40 μmol/L dCTP, dGTP, and dTTP, 20 μmol/L dATP, 2.5 μCi 1,000- to 3,000-Ci/mmol [α-^{33}P]dATP, and 10 pmol of each primer in a 50-μL vol with 50 μL mineral oil. PCR conditions included a denaturation step at 95°C for 1 minute followed by 26 cycles of 94°C for 30 seconds, 57°C for 30 seconds, and 72°C for 30 seconds. PCR products were analyzed on a 6% polyacrylamide/7 mol/L urea gel exposed to a Phospho-Imager screen suitable for quantification as described recently.[27] In this semiquantitative RT-PCR, the following 2 primer sets were used simultaneously in the same tube: the human PAI-1 gene–specific primers (301 bp) 5′-GTGTTTCAGCAGGTGGCGC-3′ sense and 5′-CCGGAACAGCCTGAAGAAGTG-3′ antisense and, as an internal standard, specific primers for the transcription factor Sp1 (230 bp), 5′-GAGAGTGGCTCACAGCCTGTC-3′ sense and 5′-GGTCAGAGCATCAGACCCCTG-3′ antisense. The sequence of the PAI-1 transcript was confirmed using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit according to the instructions of the manufacturer. To ensure that the amplification of both PAI-1 and Sp1 cDNAs was within the exponential range, different numbers of PCR cycles (20 to 28 cycles) were used. Finally, 26 cycles of PCR amplification were used for the detection of PAI-1 mRNA.

Table 1. Characteristics of the Obese and Non-Obese Subjects (mean ± SD)

Characteristic	Obese	Non-Obese	*P*
Sex ratio (male/female)	3/8	5/2	
Age (yr)	39.7 ± 13.8	41.3 ± 13.9	NS
BMI (kg/m^2)	44.8 ± 8.7	24.2 ± 2.7	<.01
Fat cell diameter (μm)			
Subcutaneous	124.0 ± 16.3	79.9 ± 13.2	<.01
Omental	106.6 ± 15.9	70.9 ± 15.4	<.01
Fat cell volume (pL)			
Subcutaneous	1,053.3 ± 426.9	286.3 ± 140.9	<.01
Omental	658.8 ± 281.6	210.1 ± 145.7	<.01

Statistical Analysis

Results are expressed as the mean ± SEM. The n value represents the number of separate experiments. Differences between groups were tested using the nonparametric Wilcoxon test. Differences with a *P* value less than .05 were regarded as significant.

RESULTS

Secretion of PAI-1 Protein by Subcutaneous and Omental Fat Cells

PAI-1 protein in the culture medium due to secretion from adipocytes was already detectable after a 2-hour incubation period in some cultures, but not all. The PAI-1 antigen concentration in the culture medium increased steadily in a time-dependent manner (culture medium of subcutaneous adipocytes: after 2 hours of incubation, 5.4 ± 0.3 ng/10^6 fat cells; after 24 hours of incubation, 80.5 ± 40.1 ng/10^6 fat cells, n = 3; culture medium of omental adipocytes: after 2 hours of incubation, 5.5 ± 0.6 ng/10^6 fat cells; after 24 hours of incubation, 153 ± 51.2 ng/10^6 fat cells, n = 3).

To compare PAI-1 protein release from isolated adipocytes of obese and non-obese individuals, we used subcutaneous and omental adipose tissue samples from 11 obese and 7 non-obese individuals. In the obese subjects, the PAI-1 antigen concentration in the medium was 2-fold higher in the cultures of omental

fat cells compared with subcutaneous fat cells (omental adipocytes, 125.5 ± 35.8 ng/10^6 fat cells/24 h; subcutaneous adipocytes, 59.6 ± 11.6 ng/10^6 fat cells/24 h, $P < .05$).

In the two obese subjects with type 2 diabetes treated with diet and metformin, the PAI-1 antigen concentration in the medium was lower than the level in the other 9 obese subjects in both subcutaneous and omental adipocytes (subcutaneous adipocytes 40.8 ± 5.6 and omental adipocytes 95.2 ± 28.1 ng/10^6 fat cells/24 h with metformin treatment; subcutaneous adipocytes 63.7 ± 13.8 and omental adipocytes 132.2 ± 43.2 ng/10^6 fat cells/24 h without diabetes).

In the 7 non-obese subjects, the amount of PAI-1 protein in the medium was also significantly higher in omental versus subcutaneous fat cells (omental adipocytes, 50.5 ± 12.6 ng/10^6 fat cells/24 h; subcutaneous adipocytes, 27.4 ± 8.9 ng/10^6 fat cells/24 h, $P < .05$). The statistical comparison between obese and lean donors also showed significantly higher PAI-1 secretion rates in both subcutaneous and omental adipocytes obtained from obese subjects (Fig 1). These differences in PAI-1 secretion between obese and non-obese subjects and between subcutaneous and omental adipocytes remained significant when the respective difference in fat cell volume was considered (data not shown).

The PAI-1 mRNA level was analyzed in subcutaneous adipose tissue samples from 7 obese and 7 non-obese subjects. The PAI-1 mRNA content detected in the subcutaneous adipose tissue depot from obese subjects was 2.5-fold higher than that detected in adipose tissue from non-obese subjects (0.38 ± 0.02 *v* 0.15 ± 0.01 PAI-1/Sp1 mRNA ratio, $P < .05$) (Fig 2).

Effect of TNF-α and TGF-β1 on PAI-1 Secretion

It is known from animal studies that PAI-1 production is stimulated by the two cytokines TNF-α and TGF-β1.[9,10,17,18,20] To examine a possible regulatory role of both cytokines in PAI-1 production in human adipocytes, we incubated fat cells from the two depots with either TNF-α or TGF-β1.

When 1 nmol/L TNF-α was added to the culture medium of adipocytes from 7 obese subjects for 24 hours, a slight but statistically nonsignificant increase of PAI-1 in the culture medium was observed in comparison to the medium without TNF-α. The increase of PAI-1 in the medium compared with

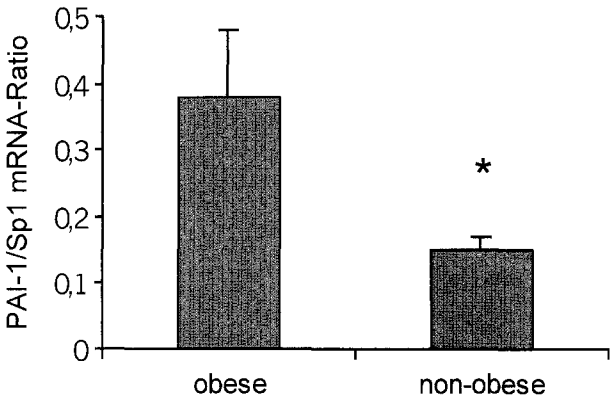

Fig 2. PAI-1 mRNA level in subcutaneous adipose tissue from obese (n = 7) and non-obese (n = 7) subjects. Data represent the mean ± SEM. *$P < .01$.

control cultures was 30% ± 21% for subcutaneous fat cells and 17% ± 18% for omental cells, respectively (each nonsignificant [NS]) (Fig 3). There also was no significant difference between the effect of TNF-α on PAI-1 release from subcutaneous and omental adipocytes.

TGF-β1 induced an increase of PAI-1 protein release into the culture medium in a dose-dependent manner. A modest increase in PAI-1 antigen accumulation (43% ± 34% of control, NS) was already observed at a concentration of 4 pmol/L (Fig 4). When 400 pmol/L TGF-β1 was present in the culture medium of adipocytes from 8 obese subjects for 24 hours, the secretion of PAI-1 protein was markedly stimulated both in subcutaneous and omental fat cells. The increase of PAI-1 induced by TGF-β1 was 87% ± 26% ($P < .05$) in fat cells from the subcutaneous adipose tissue depot and 91% ± 13% ($P < .05$) in cells from omental adipose tissue as compared with control cultures (Fig 5A). There was no significant different in the TGF-β1 effect on PAI-1 antigen secretion into the culture medium between subcutaneous and omental adipocytes. A similar stimulation of PAI-1 secretion was observed when adipocytes from 6 non-obese subjects were exposed for 24 hours to 400 pmol/L TGF-β1: PAI-1 increased 122% ± 38% ($P < .05$) in subcutaneous fat cells and 105% ± 56% (NS) in omental fat cells, respectively (Fig 5B).

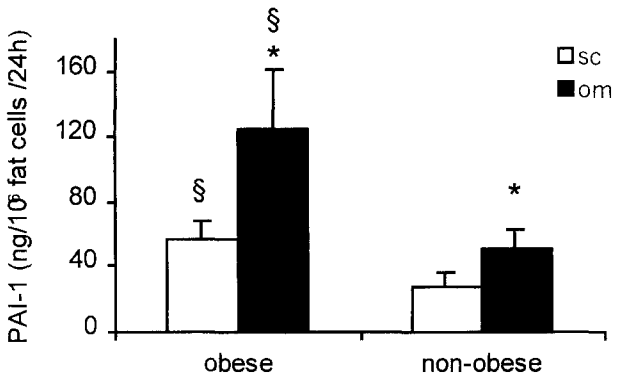

Fig 1. PAI-1 secretion from subcutaneous (SC) and omental (OM) fat cells from obese (n = 11) and non-obese (n = 7) subjects during a 24-hour culture period. Data represent the mean ± SEM. *$P < .05$ *v* SC fat cells, §$P < .05$ *v* adipocytes from non-obese subjects.

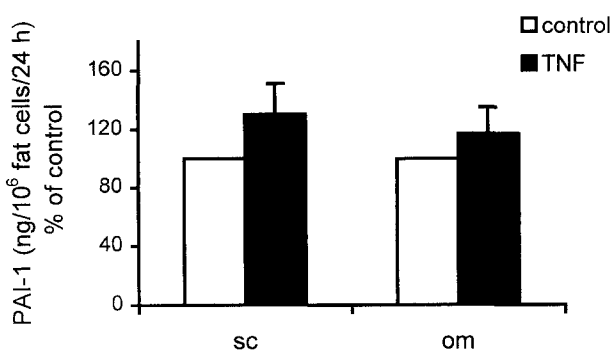

Fig 3. Effect of 1 nmol/L TNF-α on PAI-1 secretion in the conditioned medium of isolated human adipocytes from 7 extremely obese subjects. Data represent the mean ± SEM of separate experiments and are expressed as a percent of the control value (defined as 100%).

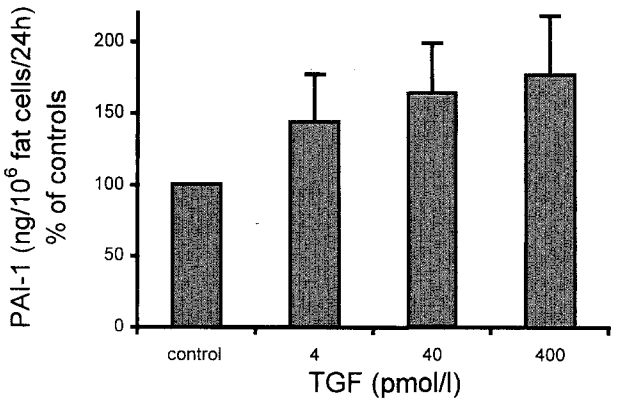

Fig 4. Effect of increasing concentration of TGF-β1 on PAI-1 protein release into the conditioned medium of isolated human omental adipocytes incubated 24 hours. Data represent the mean ± SEM of 3 separate experiments and are expressed as a percent of the control value (defined as 100%).

DISCUSSION

In this study, we analyzed the production of PAI-1 in human fat cells using an in vitro model of freshly isolated adipocytes from subcutaneous and omental adipose tissue maintained for 24 hours in suspension culture. The results clearly indicate that omental adipocytes release significantly more PAI-1 in vitro than subcutaneous adipocytes from obese and non-obese subjects, even after consideration of the differences in fat cell size. These results are in agreement with those of Alessi et al,[20] who first demonstrated a higher production of PAI-1 in omental versus subcutaneous adipose tissue from non-obese subjects. In a very recent study, Cigolini et al[23] also showed that omental adipose tissue fragments release significantly more PAI-1 into the medium than subcutaneous adipose tissue from the same obese donor. In addition, we provide evidence that this depot-specific difference is also true in severely obese subjects. By comparing isolated adipocytes from the two depots, it is possible to exclude the possibility that other cell types present in adipose tissue such as endothelial cells are responsible for PAI-1 production.

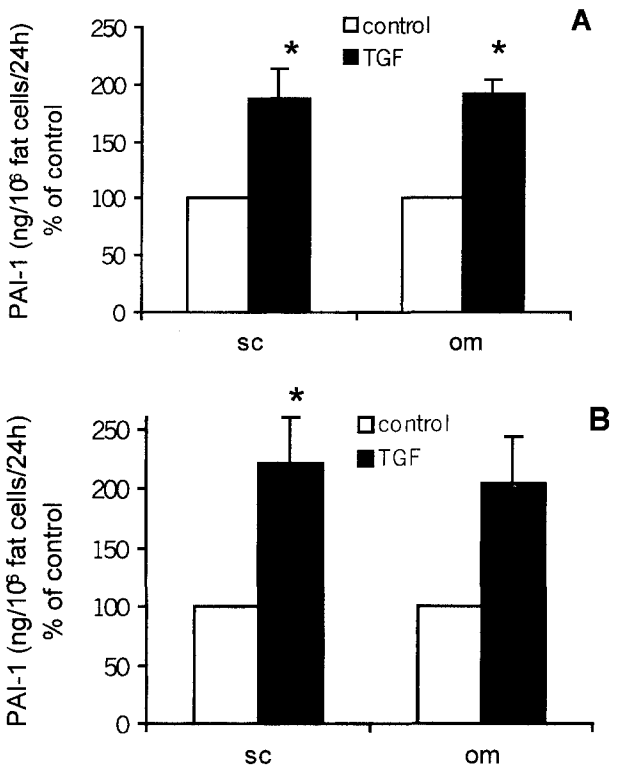

Fig 5. Effect of 400 pmol/L TGF-β1 on PAI-1 secretion from subcutaneous (SC) abdominal and omental (OM) fat cells, respectively, in comparison to control cultures. (A) Obese individuals (n = 8); (B) Non-obese individuals (n = 6). Data represent the mean ± SEM increase *v* controls during a 24-hour incubation period. *$P < .05$ *v* control values (defined as 100%).

The greater release of PAI-1 antigen from omental as compared with subcutaneous fat cells is currently unexplained. Both depots show substantial differences with regard to capillary density, innervation, and cellular composition in general. However, it is still unclear if and which of these factors are involved in the regulation of PAI-1 production and secretion from adipocytes. Since the depot-specific difference in PAI-1 secretion is observed in pure fat cell fractions after 24 hours of suspension culture, it may be speculated that this regional difference may originate from inherent differences in fat cell function.

Our data also suggest that PAI-1 antigen release from fat cells is significantly higher in obese versus non-obese individuals. This finding is also substantiated by the higher PAI-1 mRNA content in adipose tissue from obese individuals as compared with lean controls. Similar data were recently reported by Eriksson et al,[21] who also showed a positive correlation between PAI-1 secretion and fat cell volume in non-obese individuals. Thus, the elevated plasma concentration of PAI-1 in obese subjects may be at least partially explained by an increased release from large fat cells, as well as expansion of the total fat mass, particularly if the growth of adipose tissue is preferentially located in the omental depot. In our study, an ELISA technique was used to quantify PAI-1 antigen release from adipose tissue. As the role of PAI-1 in fibrinolysis depends on its activation,[28] it is important to know the extent to which PAI-1 is present in its active form in the culture medium. In agreement with previous reports from in vitro studies,[29,30] PAI-1 activity was at the detection level in our culture system (Gottschling-Zeller et al, unpublished data, 1999). However, one must note that the culture medium is serum-free and PAI-1 activation cannot occur under these conditions.

It was recently shown that the antidiabetic agent metformin reduces PAI-1 levels in women with polycystic ovary syndrome. However, most of the patients in these studies were not diabetic.[31,32] It is noteworthy that fat cells from the two obese type 2 diabetic subjects treated with metformin released comparably lower amounts of PAI-1 protein. This circumstantial finding may indicate that metformin also reduces PAI-1 synthesis/release from adipocytes. However, this assumption should be examined by clinical studies directly addressing this issue.

Another aim of this study was to examine the effect of agents known to influence PAI-1 production. We particularly investigated the effect of TGF-β1 and TNF-α on PAI-1 release by fat cells. Previous studies in various cell types in vitro and murine tissues in vivo have shown a stimulatory effect of both

cytokines on PAI-1 biosynthesis.[13,16,18] In the first such study in human adipose tissue, Alessi et al[20] did not find an effect of TNF-α on PAI-1 production in explants of human adipose tissue. In a similar study in murine 3T3-L1 adipocytes, Lundgren et al[33] also did not observe an effect of TNF-α, whereas Samad et al[18] reported an increase of PAI-1 mRNA expression in adipose tissue of TNF-α–treated mice. Very recently, Cigolini et al[23] demonstrated that an 8-hour incubation of subcutaneous and omental adipose tissue fragments with TNF-α produced a significant increase of PAI-1 protein release into the medium. In the same study, treatment of adipose tissue fragments with 10 to 40 ng/mL TNF-α increased PAI-1 mRNA already after 4 hours of incubation.[23] However, since tissue fragments were used, it is possible that cells other than adipocytes contributed to this response. In our model, we found only a moderate stimulation of PAI-1 secretion after incubation with TNF-α in omental and subcutaneous fat cells from obese subjects. Nevertheless, this finding may support the idea that the chronic elevation of TNF-α production in the obese state[22] could stimulate and maintain an elevated PAI-1 production and secretion in human adipocytes.

We also studied the effect of the multifunctional cytokine TGF-β1 on PAI-1 release by subcutaneous and omental fat cells and observed a marked stimulation of PAI-1 antigen release in both depots independently of the BMI of the donor. Previous studies have clearly shown that TGF-β1 increases PAI-1 gene expression in cultured 3T3-L1 adipocytes,[24,33] as well as human adipose tissue.[20] Infusion of TGF-β1 in mice induced PAI-1 mRNA, especially in adipose tissue.[13] There is indirect evidence that this effect may be of physiological importance, as a recent study demonstrated an overexpression of TGF-β1 in adipose tissue from genetically obese mice.[19] It is presently unclear whether TGF-β1 is also expressed in human adipose tissue and, if so, whether TGF-β1 overexpression is present in human obesity, which could then contribute to explain the increased plasma PAI-1 levels observed in this condition.

In conclusion, the results of this study indicate that human fat cells secrete substantial amounts of PAI-1 that may contribute to the elevated plasma concentration and increased thromboembolic risk in obese patients. Moreover, omental fat cells are characterized by a higher rate of secretion of PAI-1 than subcutaneous fat cells. Our experiments on the regulation of PAI-1 release suggest that TGF-β1 is an important physiological promoter in human adipocytes from both depots and from both obese and lean subjects.

ACKNOWLEDGMENT

We wish to thank Professor B. Husemann and his team from the Surgical Department, Dominikus-Krankenhaus, Düsseldorf-Heerdt, for their support in obtaining adipose tissue samples.

REFERENCES

1. Kissebah AH, Krakower GR: Regional adiposity and morbidity. Physiol Rev 74:761-811, 1994

2. Juhan-Vague I, Alessi MC: PAI-1, obesity, insulin resistance and risk of cardiovascular events. Thromb Haemost 78:656-660, 1997

3. Hekman CM, Loskutoff DJ: Kinetic analysis of the interactions between plasminogen activator inhibitor 1 and both urokinase and tissue plasminogen activator. Arch Biochem Biophys 2625:199-210, 1988

4. Wiman B, Chmielewska J, Ranby M: Inactivation of tissue plasminogen activator in plasma. Demonstration of a complex with a new rapid inhibitor. J Biol Chem 259:3644-3647, 1984

5. McGill J, Schneider DJ, Arfken CL, et al: Factors responsible for impaired fibrinolysis in obese subjects and NIDDM patients. Diabetes 43:104-109, 1994

6. De Pergola G, De Mitrio V, Scaraffia M, et al: Lower androgenicity is associated with higher plasma levels of prothrombotic factors irrespective of age, obesity, body fat distribution, and related metabolic parameters in men. Metabolism 46:1287-1293, 1997

7. De Pergola, De Mitrio V, Giorgino F, et al: Increase in both pro-thrombotic and anti-thrombotic factors in obese premenopausal women: Relationship with body fat distribution. Int J Obes Relat Metab Disord 21:527-535, 1997

8. Janand-Delenne B, Chaugnaud C, Raccah D, et al: Visceral fat as a main determinant of plasminogen activator inhibitor 1 level in women. Int J Obes Relat Metab Disord 22:312-317, 1998

9. Vague P, Juhan-Vague I, Ailhaud MF, et al: Correlation between blood fibrinolytic activity, plasminogen activator inhibitor level, plasma insulin level, and relative body weight in normal and obese subjects. Metabolism 35:250-253, 1986

10. Vague P, Juhan-Vague I, Chabert V, et al: Fat distribution and plasminogen activator inhibitor activity in nondiabetic obese women. Metabolism 38:913-915, 1989

11. Gray RP, Panahollo A, Mohamed-Ali V, et al: Proinsulin-like molecules and plasminogen activator inhibitor type 1 (PAI-1) activity in diabetic and non-diabetic subjects with and without myocardial infarction. Atherosclerosis 130:171-178, 1997

12. Juhan-Vague I, Pyke SDM, Alessi MC, et al: Fibrinolytic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. Circulation 94:2057-2063, 1996

13. Sawdey MS, Loskutoff DJ: Regulation of murine type 1 plasminogen activator inhibitor gene expression in vivo. Tissue specificity and induction by lipopolysaccharide, tumor necrosis factor-α, and transforming growth factor-β. J Clin Invest 88:1346-1353, 1991

14. Schneider DJ, Sobel BE: Augmentation of synthesis of plasminogen activator inhibitor type 1 by insulin and insulin-like growth factor type 1: Implications for vascular disease in hyperinsulinemic states. Proc Natl Acad Sci USA 88:9959-9963, 1991

15. Schneider DJ, Nordt TK, Sobel BE: Attenuated fibrinolysis and accelerated atherogenesis in type II diabetic patients. Diabetes 42:1-7, 1993

16. van Hingsberg VW, Kooistra T, van den Berg EA, et al: Tumor necrosis factor increases the production of plasminogen activator inhibitor in human endothelial cells in vitro and in rats in vivo. Blood 72:1467-1473, 1988

17. Shimomura I, Funahashi T, Takanashi M, et al: Enhanced expression of PAI-1 in visceral fat: Possible contributor to vascular disease in obesity. Nat Med 2:800-803, 1996

18. Samad F, Yamamoto K, Loskutoff DJ, et al: Distribution and regulation of plasminogen activator inhibitor-1 in murine adipose tissue in vivo. Induction by tumor necrosis factor-α and lipopolysaccharide. J Clin Invest 97:37-46, 1996

19. Samad F, Yamamoto M, Pandey M, et al: Elevated expression of transforming growth factor-β in adipose tissue from obese mice. Mol Med 3:37-48, 1997

20. Alessi MC, Pirelli F, Morange P, et al: Production of plasminogen activator inhibitor 1 by human adipose tissue. Possible link between visceral fat accumulation and vascular disease. Diabetes 46:860-867, 1997

21. Eriksson P, Reynisdottir S, Lönnqvist F, et al: Adipose tissue

secretion of plasminogen activator inhibitor-1 in non-obese and obese individuals. Diabetologia 41:65-71, 1998

22. Hotamisligil GS, Shargill NS, Spiegelman BM: Adipose expression of tumor necrosis factor-α: Direct role in obesity-linked insulin resistance. Science 259:87-91, 1993

23. Cigolini M, Tonoli M, Borgato L, et al: Expression of plasminogen activator inhibitor-1 in human adipose tissue: A role for TNF-α? Atherosclerosis 143:81-90, 1999

24. Fearns C, Loskutoff DJ: Induction of plasminogen activator inhibitor 1 gene expression in murine liver by lipopolysaccharide. Cellular localization and role of endogenous tumor necrosis factor-α. Am J Pathol 150:579-590, 1997

25. Rodbell M: Metabolism of isolated fat cell. I. Effects of hormones on glucose metabolism and lipolysis. J Biol Chem 239:375-380, 1964

26. Chomczynski P, Sacchi N: Single-step method for isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156-159, 1987

27. Wabitsch M, Jensen PB, Blum WF, et al: Insulin and cortisol promote leptin production in cultured human fat cells. Diabetes 45:1435-1438, 1996

28. Loskutoff DJ, Samad F: The adipocytes and hemostatic balance in obesity. Studies of PAI-1. Arterioscler Thromb Vasc Biol 18:1-6, 1998

29. Hekman CM, Loskutoff DJ: Endothelial cells produce a latent inhibitor of plasminogen activators that can be activated by denaturants. J Biol Chem 260:11581-11587, 1985

30. Nordt TK, Schneider DJ, Sobel BE: Augmentation of the synthesis of plasminogen activator inhibitor type-1 by precursors of insulin. A potential risk factor for cardiovascular disease. Circulation 89:321-330, 1994

31. Velasquez EM, Mendoza SG, Wang P, et al: Metformin therapy is associated with a decrease in plasma plasminogen activator inhibitor-1. Lipoprotein(a) and immunoreactive insulin levels in patients with polycystic ovary syndrome. Metabolism 46:454-457, 1997

32. Glueck CJ, Wang P, Fontaine R, et al: Metformin-induced resumption of normal menses in 39 of 43 (91%) previously amenorrheic women with polycystic ovary syndrome. Metabolism 48:511-519, 1999

33. Lundgren CH, Brown SL, Nordt TK, et al: Elaboration of type-1 plasminogen activator inhibitor from adipocytes. A potential pathogenic link between obesity and cardiovascular disease. Circulation 93:106-110, 1996